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Minireview

A Model for the Catalytic Site of F_1-ATPase Based on Analogies to Nucleotide-Binding Domains of Known Structure

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An updated topological model is constructed for the catalytic nucleotide-binding site of the F_1-ATPase. The model is based on analogies to the known structures of the MgATP site on adenylate kinase and the guanine nucleotide sites on elongation factor Tu (Ef-Tu) and the *ras* p21 protein. Recent studies of these known nucleotide-binding domains have revealed several common functional features and similar alignment of nucleotide in their binding folds, and these are used as a framework for evaluating results of affinity labeling and mutagenesis studies of the β subunit of F_1. Several potentially important residues on β are noted that have not yet been studied by mutagenesis or affinity labeling.

KEY WORDS: F_1-ATPase; nucleotide binding sites; protein folding; structure prediction.

INTRODUCTION

There are a total of six nucleotide-binding sites on F_1-ATPases from bovine mitochondria (Cross and Nalin, 1982), *Escherichia coli* (Wise *et al.*, 1983), and spinach chloroplasts (Xue *et al.*, 1987; Girault *et al.*, 1988). Three of the sites exchange bound nucleotide rapidly during catalytic turnover and are potential catalytic sites, whereas the three remaining sites have no known function and are referred to as noncatalytic sites (Cross, 1988). Isolated α and β subunits from *E. coli* F_1 each contain a single nucleotide-binding site (Dunn and Futai, 1980; Rao *et al.*, 1988a). The properties of these sites suggest that catalytic sites are on β subunits and noncatalytic sites on α (Senior, 1988). The presence of α and β in F_1 in three copies each (Foster and Fillingame, 1982) and the finding that α and β arose by gene duplication (Walker *et al.*, 1982) lead to the long-held belief that noncatalytic sites were entirely on α and catalytic sites entirely on β subunits. However, more recent evidence suggests that at least the noncatalytic sites are positioned at α–β subunit interfaces (Bullough and Allison, 1986b; Kironde and Cross, 1987; Cross *et al.*, 1987; Lunardi *et al.*, 1987; Verburg and Allison, 1990; Zhuo *et al.*, 1992).

When noncatalytic sites are loaded with FSBA[2] or 2-azido-ANP, a single specific tyrosine is labeled. Similarly, with FSBI or 2-azido-ANP loaded at catalytic sites, a different specific tyrosine is labeled (Bullough and Allison, 1986b; Cross *et al.*, 1987). Kinetic and isotope exchange experiments provide evidence for three functional catalytic sites (Cross *et al.*, 1982; Xue *et al.*, 1988). Taken together, these results provide strong support for the presence of two different types of sites, in three copies each. However, other suggestions have been made (Berden *et al.*, 1991; Shapiro *et al.*, 1991).

Affinity labeling and mutagenic studies have identified a number of α and β residues that affect nucleotide binding and/or catalysis. However, interpret-

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[2]Abbreviations: AK, adenylate kinase; ANP, ADP or ATP; A_p 4A, 5′5′-diadenosine tetraphosphate; DCCD, dicyclohexylcarbodiimide; EF-Tu, elongation factor Tu of *E. coli*; F_1, the extrinsic, dissociable sector of the H^+-translocating ATP synthase; FSBA, 5′-*p*-fluorosulfonylbenzoyladenosine; FSBεA, 5′-*p*-fluorosulfonylbenzoyl-1,N^6-ethenoadenosine; FSBI, 5′-*p*-fluorosulfonylbenzoylinosine; Nbf-Cl, 7-chloro-4-nitrobenzofurazan; PLP-AMP and PLP-ADP, pyridoxal 5′-di- and triphosphoadenosine; Ras, *ras* p21 protein.

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ing the roles of such residues is difficult in the absence of a high-resolution structure. To help evaluate information regarding catalytic sites, earlier studies drew analogies to the known structures of adenylate kinase (AK) and other nucleotide-binding proteins which show limited regions of sequence homology with the β subunit (Duncan *et al.*, 1986; Fry *et al.*, 1986). Based on NMR (Fry *et al.*, 1985, 1987) and X-ray crystallographic (Egner *et al.*, 1987) structures for the ATP and AMP sites on AK, this analogy was extended to include both catalytic and noncatalytic nucleotide sites on F_1, with the noncatalytic site positioned at an α–β subunit interface (Cross *et al.*, 1987; Penefsky and Cross, 1991). In these models, adjacent catalytic and noncatalytic sites are positioned like the ATP and AMP sites of AK, with the terminal phosphoryl groups in close proximity. This model is supported by the finding that AP_4A, a potent inhibitor of AK, also inhibits F_1 (Vogel and Cross, 1991). In contrast, Zhuo *et al.* (1992) proposed that the adenine rings of nucleotides bound at adjacent sites interact near the α–β subunit interface, with the phosphoryl chains farthest apart in the separate subunits. The proximity of catalytic and noncatalytic sites, a feature of both models, is supported by studies with spin-labeled nucleotides which indicate that the sites lie within $\sim$15 Å of each other (Vogel-Claude *et al.*, 1988; Vogel *et al.*, 1992). However, this view is at odds with fluorescence resonance energy transfer studies on chloroplast F_1 which indicate that no two sites are closer than $\sim$35 Å (Shapiro *et al.*, 1991).

In this review, we update the analogies between F_1 catalytic sites and nucleotide-binding folds on AK, *E. coli* elongation factor Tu (EF-Tu) and the *ras* p21 proteins (Ras). Topological and functional constraints implied from the known structures are used as interim guideposts for interpreting biochemical and genetic studies of the nucleotide-binding sites of F_1. Since the majority of mutagenic studies have been done on the *E. coli* system, we use residue numbers for *E. coli* β subunit unless otherwise noted.

TOPOLOGICAL ANALOGIES TO ADENYLATE KINASE, ELONGATION FACTOR Tu, AND Ras

The sequence of *E. coli* β subunit is given in Fig. 1. The elements of predicted secondary structure shown above the sequence are similar to those from earlier predictions for *E. coli* β (Walker *et al.*, 1984) and rat liver mitochondrial β (Garboczi *et al.*, 1988) except that only the most strongly predicted "core" elements are given. Two regions of sequence homology with other nucleotide-binding proteins recognized by Walker *et al.* (1982) are denoted by asterisks below the sequence. Sequence A (149–156) is GXXXXGK (-)(T/S) where X is any residue. This sequence follows β-strand 1 in AK and EF-Tu, and includes the P-loop motif subsequently found in a variety of purine nucleotide-binding proteins (Saraste *et al.*, 1990). Sequence B (231–242) is (R/K)X(-) XGXXXLZZZ$(X)_{0-2}$D, where each Z is a nonpolar residue. The nonpolar residues span β-strand 4 in AK and EF-Tu. the doubly wound $\alpha\beta$ folding topologies (Richardson, 1981) of porcine AK (Dreusicke *et al.*, 1988) and EF-Tu (Clark *et al.*, 1990) both have a central "core" β-sheet with H-bonded parallel strands in the order 5-4-1-3, and the proximity of sequences A and B within this core was a major determinant in the original topological modeling of the F_1 β subunit (Duncan *et al.*, 1986). Several recent findings strongly support the idea that this core β-sheet is conserved in numerous nucleotide-binding proteins. The folding topologies of yeast guanylate kinase (Stehle and Schulz, 1990) and Ras (Wittinghofer and Pai, 1991) were found to be identical to those of AK and EF-Tu, respectively, although each pair of proteins shows only 17% sequence identity. Furthermore, the P-loop conformations are essentially superimposable for AK, EF-Tu, and Ras (Saraste *et al.*, 1990; Chen *et al.*, 1990), and a core of more than 100 residues throughout β-strands 5-4-1-3 and their neighboring α-helices show close α-carbon alignments between porcine AK and EF-Tu (Milner-White *et al.*, 1991). Thus, for the F_1 β subunit, an analogous core of parallel H-bonded strands 5-4-1-3 and their neighboring α-helices remains a reasonable prediction for topological modeling. Figure 2 shows a schematic model for the catalytic site on β with the core β-sheet emphasized by bold lines.

Greater variations occur in elements near the "sides" of the doubly wound $\alpha\beta$ fold in known structures. Only EF-Tu and Ras protein have a sixth parallel β-strand that H-bonds with β-strand 5 on the left (Fig. 2). In contrast to AK, EF-Tu and Ras do not contain a second nucleotide site and simply have a "hairpin" connection between antiparallel β-strand 2 and β-strand 3 on the right. In Fig. 2, β-strand 2 is shown without a directional arrow or connections to strands 1 and 3, to indicate that it may be parallel or antiparallel. However, β has insufficient sequence between predicted β-strands 2 and 3 to form a cluster of α-helices such as that found at the AMP site of AK. Thus, if the

noncatalytic site of F_1 is oriented like the AMP site of AK, then α-subunit residues must make significant contributions to the site. This is consistent with ligand binding and affinity labeling studies noted above.

FUNCTIONAL ELEMENTS OF AK, EF-Tu, AND Ras NUCLEOTIDE-BINDING DOMAINS: IMPLICATIONS FOR $F_1\beta$ SUBUNITS

Recent crystallographic studies of several members of the AK family (Egner *et al.*, 1987; Stehle and Schulz, 1990; Diedrichs and Schulz, 1991) and mutagenic studies of AK from several species (reviewed by Tsai and Yan, 1991) have resulted in a revised model for the ATP, Mg^{2+}, and AMP binding sites. In light of these results, it is apparent that the MgATP binding site on AK shares several common features with the MgGDP/GTP binding sites of EF-Tu (Clark *et al.*, 1990) and Ras (Wittinghofer and Pai, 1991). In each case, nucleotide in the *anti* conformation straddles the carboxyl end (top edge, Fig. 2) of the parallel β-sheet. The purine ring makes numerous contacts with loops and turns following β-strands 4 and 5 (and 6 in EF-Tu, Ras), whereas the ribose appears to have few specific interactions with the protein. The di- or triphosphate moiety extends over the P-loop and the amino end of the following α-helix 1. The γ-phosphoryl and bound Mg^{2+} are oriented toward the carboxyl ends of β-strands 3 and 2. The general features of the Mg^{2+} and phosphoryl binding domains are apparent even in the nucleotide-binding folds of more divergent structures such as the *recA* protein (Story and Steitz, 1992).

Using the model in Fig. 2 for the catalytic site of F_1, we now consider β residues studied by mutagenesis and affinity labeling and their possible correlation with known features of AK, EF-Tu, and Ras. Since the nucleotide-binding domains of EF-Tu and Ras are very similar and current structural data on Ras is more detailed, only representative features of Ras are discussed. Information on Ras and AK is drawn from the references cited above unless noted otherwise. Original references for mutagenesis and affinity labeling of β are listed in the legend to Fig. 1, whereas citations in the text refer to specific properties of mutant and labeled enzymes.

The P-loop and α-helix 1

It is interesting to note that several naturally invariant residues in the P-loop of β have been mutated without drastic effects on catalytic rates (G149I/S, G150S, G154I and the double mutant G149I + G154I), while other mutations (A151V or G152R/D) cause much greater catalytic defects. The conserved Lys of sequence A (K155) is at the amino end of α-helix 1 in AK and Ras and directly interacts with the β-phosphoryl of bound nucleotide. Mutations in the F_1 β subunit, K155Q (see Senior, this issue) and K155I (PS3 mutant), cause almost complete loss of catalysis, and, for K155Q F_1, the ~100-fold higher K_D for binding "unisite" ATP (Al-Shawi *et al.*, 1989; see also Senior, this issue) is consistent with the loss of 1 or 2 H-bonds or an electrostatic bond with the β-phosphoryl of ATP.

Near the amino end of α-helix 1, the hydroxyl of a conserved Thr (porcine AK, T23; Ef-Tu, T25) or Ser (Ras S17) also interacts with the β-phosphoryl of bound nucleotide. In Ras, it may also interact with Mg^{2+}. Takeyama *et al.* (1990) used computer modeling to place the β subunit's sequence A in the conformation of the Ras P-loop/α-helix 1 and did energy refinements to model the possible changes caused by different mutations. Their most notable prediction was that insertion of Gly before T156 would cause the Thr to be shifted to the opposite face of α-helix 1, preventing its interaction with the bound nucleotide. Indeed, F_1 with this insertion was found to be almost completely inactive. F_1 was also essentially inactive when T156 was replaced by Ala or Cys (Iwamoto *et al.*, 1991). With *Saccharomyces cerevisiae* F_1, changing T156 to Ser (like Ras S17) actually increased the maximal ATPase rate 3-fold. With *S. pombe* F_1, substitution of Tyr for the residue equivalent to M160 increased the affinity of F_1 for inhibitory ADP (Falson *et al.*, 1991). In proposed α-helix 1, T156 and M160 would share a peptide H-bond, and their side chains would be near the same face of the helix. However, in AK and Ras, the residue corresponding to M160 does not directly contact the nucleotide's base or phosphoryl groups. At the next position of proposed α-helix 1, mutations E161Q/R also cause 90/99% loss of membrane ATPase activity. In summary, this segment appears to be an important part of the substrate-binding domain.

β-Strand 2 and the Next Connecting Segment

The mutation S174F alters the Mg^{2+}/Ca^{2+} dependence of F_1 ATPase activity (Kanazawa *et al.*, 1980; Senior *et al.*, 1983), suggesting that S174 interacts with Mg^{2+}. Iwamoto *et al.* (1991) noted that

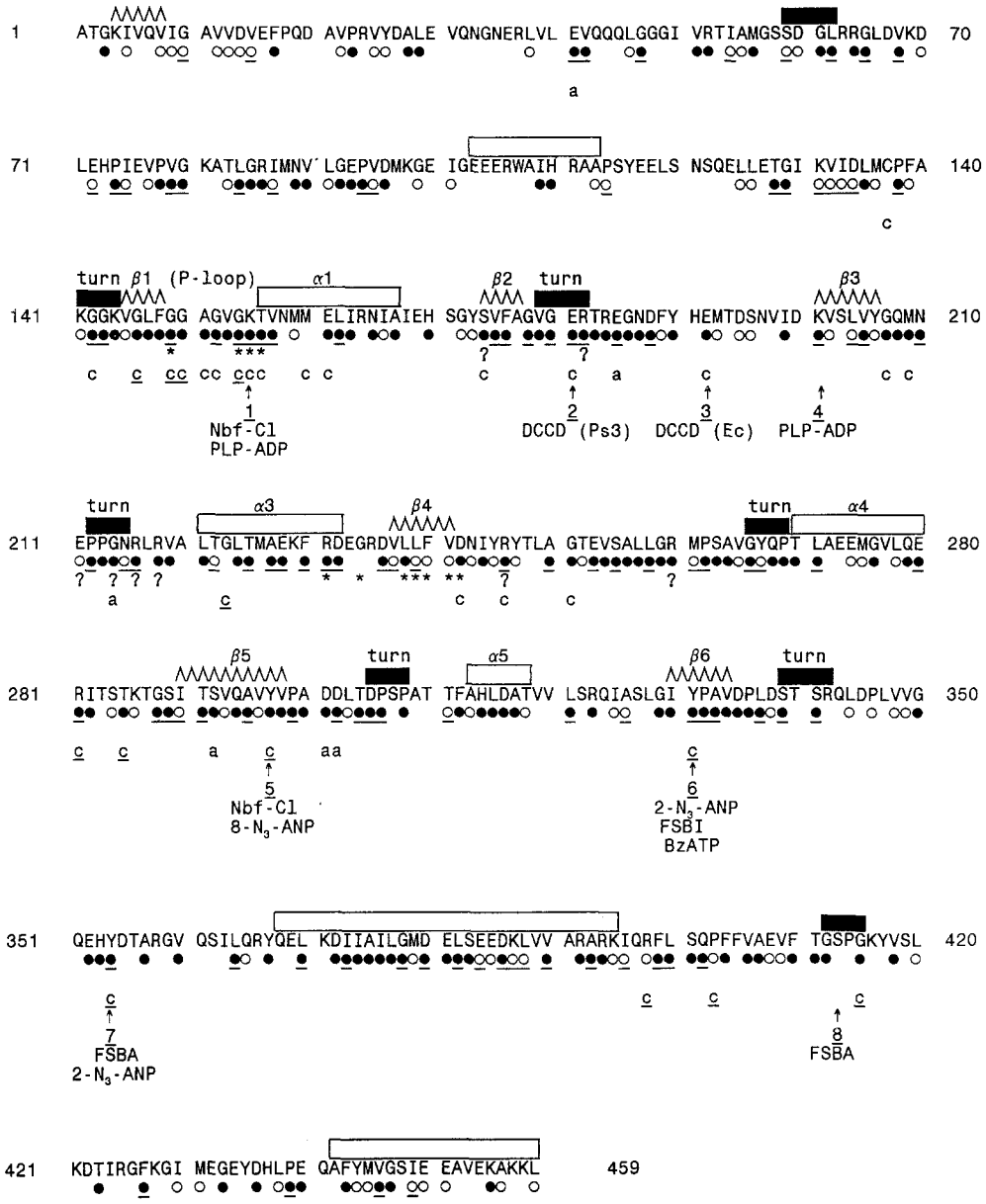

Fig. 1. The β subunit of *E. coli* F_1. The sequence is from Walker *et al.* (1984). Minimal elements of predicted secondary structure (shown above the sequence) were derived by comparing predictions made by the methods of McLachlan (see Walker *et al.*, 1984) and Garnier *et al.* (1978). Only those α-helices (□) and β-strands (ΛΛ) that are strongly predicted by both algorithms are shown here. All β-turns shown (■) are predicted by the second method, all but two by the first, and all show highly conserved residues in β of other species. The β-strands thought to form the β-sheet of the nucleotide-binding domain are labeled β1 through β6; each α-helix thought to be part of that domain is numbered after the preceding β-strand in the sequence. *Notations below the sequence*: ●, invariant residues in β of at least 15 species [5 chloroplasts, 5 mitochondrial, 5 bacterial; aligned sequences in Ysern *et al.* (1988), Penefsky and Cross (1991), and *S. pombe* β (Falson *et al.*, 1991)]; ○, residues that are identical in at least 10 species or conservative replacements in all species (note: in all other species, *E. coli* E72 and N215 are G and A, respectively); underlined circles indicate conserved residues are found in the catalytic subunit of vacuolar ATPases and archaebacterial ATP synthases (Penefsky and Cross, 1991); * denotes conserved residues of sequences A and B (Walker *et al.*, 1982); ? denotes possible analogy to functional residue in AK, Ras, or EF-Tu (see text); **a**, "assembly" defects were caused by missense mutation at that residue, **c**, "catalytic" defects were caused by missense mutations at most of these sites, while **c** indicates that at least one mutation at that site yielded enzyme with > 25% of normal ATPase activity, indicating that residue is not essential for catalysis; arrows with numbers indicate positions of covalent modification (in one or more species) by the specified reagents. Mutagenesis references for *E. coli* β: E41K, E185K, S292F, Noumi *et al.*, 1986a; C137Y, G142D,

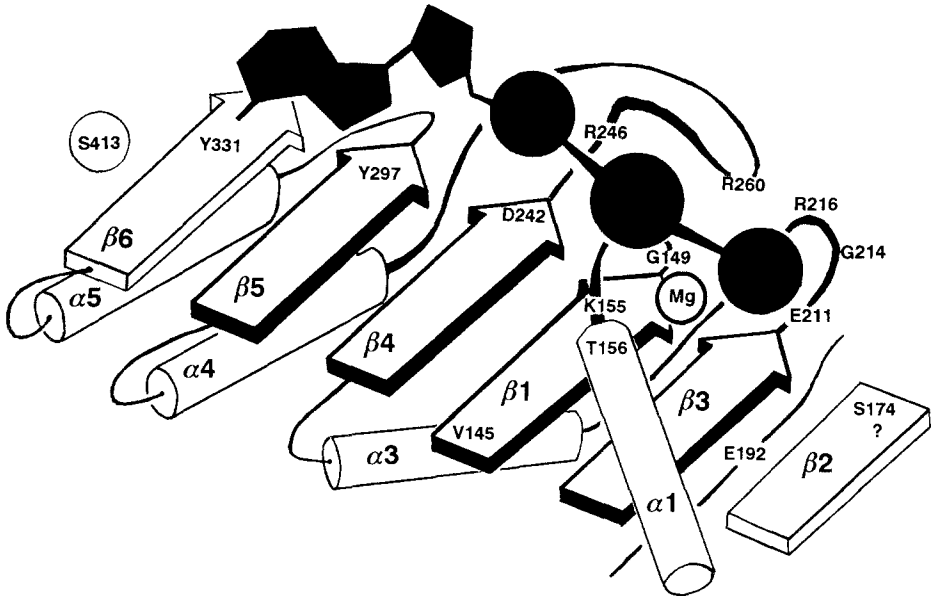

Fig. 2. Schematic model for the catalytic nucleotide site on β. Topology of secondary structural elements and the positions of bound ATP (solid black) and Mg^{2+} (circled) are in analogy to AK, EF-Tu, and Ras, as discussed in the text. The "core" β-strands (5-4-1-3) are outlined in bold. Dimensions are adjusted only for visual clarity, and some segments of protein sequence are not shown.

S174 may correspond to Ras T35, which is in a loop just before β-strand 2, and interacts with both Mg^{2+} and the γ-phosphoryl of bound GTP. The P-loop mutation G149S increases F_1-ATPase specificity for Mg^{2+} *vs* Ca^{2+}, indicating it may also affect coordination of Mg^{2+}, and this mutation partially reverses the effects of the S174F mutation when both are expressed. This functional interaction and the analogy of S174 with Ras T35 favor the antiparallel alignment of β-strand 2 with β-strand 3, placing S174 near the top of β-strand 2 in Fig. 2.

Some of the largest structural changes between the GDP- and GTP-liganded states of Ras occur in the region around Ras T35 and are thought to be related to conformational interactions of Ras with GTPase activating protein. Also, in AK, secondary structural elements in the corresponding region are thought to undergo the greatest movements upon ligand binding (Egner *et al.*, 1987). Hence, in the β subunit of F_1, β-strand 2 and nearby topological segments could be involved in conformational interactions with a neighboring α subunit. Conformational coupling between catalytic sites requires α-β intersubunit signalling (see Senior, 1988), and the importance of α is evident in the 3.6 Å structure of rat liver F_1 (Bianchet *et al.*, 1991), which shows that β subunits do not make direct contact. Following β-strand 2 are 11 invariant residues, including a strongly-predicted β-turn, and residues

G146S, G207D, G223D, P403S/G415D double mutant, Kironde *et al.*, 1989; G142S, S174F, M209I, R246C, Parsonage *et al.*, 1987a; G149I, G154I, K155E/Q, Y297F, Y354F, Parsonage *et al.*, 1987b; A151V, Hsu *et al.*, 1987; C137S, G152D/R, E161Q/R, G251D, D301V, D302V, R398C, R398W, Lee *et al*, 1991; G149S, G150S, T156A/C, Iwamoto *et al.*, 1991; E181Q, E192Q, Parsonage *et al.*, 1988; D242N/V, Al-Shawi *et al.*, 1988; R246H, T285D, Noumi *et al.*, 198000^U+988; Y331A/C/F/G/S, Wise, 1990; Y331E/L, Weber *et al.*, 1992. Mutagenesis references for PS3 β (PS3 numbers in parentheses): K155I (164), D242N (252), Yohda *et al.*, 1988; E181Q (190), E192Q (201), Ohtsubo *et al.*, 1987; Y297C/F (307), Y331C/F (341), Y354C/F (364), Odaka *et al.*, 1990. Mutagenesis references for yeast β (yeast numbers in parentheses): *S. cerevisiae*, T156S (197), R281A/K (328), Mueller, 1988, 1989; *S. pombe* 160Y(Q170), Falson *et al.*, 1991. Affinity labeling references: 1, Andrews *et al.*, 1984 (Nbf-Cl, bovine K162), Tagaya *et al.*, 1988 (PLP-ADP, *E. coli*); 2 and 3, Yoshida *et al.*, 1981, 1982; 4 (*E. coli*), Ida *et al.*, 1991; 5 (bovine Y311), Andrews *et al.*, 1984 (Nbf-Cl), Hollemans *et al.*, 1983 (8-azido-ANP); 6, Garin *et al.*, 1986 and Cross *et al.*, 1987 (2-azido-ANP, bovine Y345), Wise *et al.*, 1987 (2-azido-ANP, *E. coli*), Bullough and Allison, 1986b (FSBI, bovine), Admon and Hammes, 1987 (3′-*o*-(4-benzoyl)benzoyl-ATP, spinach); 7 (bovine Y368), Esch and Allison, 1978 (FSBA), Cross *et al.*, 1987 (2-azido-ANP); 8, Bullough and Allison, 1986a (bovine H427).

E181 and E192, which can be labeled by DCCD (Fig. 1). Data from several species indicate that inactivation by DCCD primarily disrupts catalytic cooperativity (Tommasino and Capaldi, 1985; Kandpal *et al.*, 1985; Melese and Boyer, 1985), again consistent with a possible role for this region in conformational transitions. Mutation E181Q causes a greater impairment of unisite and multisite catalysis than does the E192Q mutation (Al-Shawi *et al.*, 1989, 1990). Involvement of this region in intersubunit contact is further supported by the "assembly" defects caused by the E185K/Q mutations (Noumi *et al.*, 1986a, 1987).

The corresponding region of the α subunit may also be involved in α–β subunit interactions. In predicted β-strand 2, αC193 is close to an interface with a β subunit, as shown by mercurial labeling in the 3.6 Å map of rat liver F_1 (Bianchet *et al.*, 1991; "site 1"). Interaction of this side of the α subunit's β-sheet with the equivalent region of the β subunit could provide the juxtaposition of catalytic and noncatalytic nucleotides proposed by Penefsky and Cross (1991), with the phosphoryl groups of each near the interface.

Just after β-strand 2 in the β subunit, R182 was proposed (Penefsky and Cross, 1991) to correspond to R44 of porcine AK, which interacts with phosphate of bound AMP. In the α subunit, αK201 is equivalent to βR182 and is labeled by PLP-AMP in isolated α subunit (Rao *et al.*, 1988b), indicating αK201 is at a noncatalytic site. Tagaya *et al.* (1988) labeled αK201 with PLP-ADP in intact F_1 and argued that αK201 is near the γ-phosphoryl of ATP bound at a catalytic site, although they did not rule out labeling of noncatalytic sites. In the arrangement of catalytic and noncatalytic sites proposed by Penefsky and Cross (1991), both βR182 and αK201 are at the α–β interface, and αK201 might be labeled by PLP-ADP bound at either site.

β-Strand 3 and a Conserved Turn or Loop

By analogy with AK and Ras, this portion of β may be intimately involved in catalytic events and conformational coupling. Near the carboxyl end of β-strand 3, an Asp carboxylate is involved in coordinating Mg^{2+} in AK (porcine D93) and Ras (D57). In Ras and EF-Tu, this Asp is part of a consensus sequence, DXXG, where XXG is part of a conserved β-turn; Ras G60 appears to form an amide H-bond with an oxygen of the γ-phosphoryl of bound GTP or GMPPNP, and the next residue, Ras Q61, may participate in the hydrolytic step by H-bonding to the nucleophilic water molecule. The β-turn containing Ras G60 and Q61 is part of the second major element that undergoes conformational changes between the GDP/GTP-liganded states, and the conformational changes appear to be linked to loss of H-bond between G60 and the γ-phosphoryl upon hydrolysis. In AK, the Asp involved in coordinating Mg^{2+} also precedes a conserved β-turn, and R97 in that turn appears to be near the site of transphosphorylation, interacting specifically with the phosphate of bound AMP (Tsai and Yan, 1991). In the β subunit of F_1, residues 211–214 (EPPG) might correspond to the DXXG consensus of Ras and EF-Tu and E211 could be involved in coordinating Mg^{2+}. Following the β-turn, R216 or R218 could function like Ras Q61, H-bonding to water, or like AK R97, interacting with a phosphoryl group of a neighboring noncatalytic nucleotide. Residues 211–218 have not yet been studied by mutagenesis, but the dramatic effects of the nearby M209I mutation on catalysis (Duncan and Senior, 1985) are consistent with an important function for this region. K201, at the amino end of β-strand 3, is labeled by PLP-ADP only in the presence of Mg^{2+} (Ida *et al.*, 1991). Movement of this Lys side chain toward the phosphoryls of bound catalytic nucleotide in the presence of Mg^{2+} could be related to conformational changes in neighboring structural elements as discussed above.

β-Strand 4 through α-Helix 4

In AK and Ras, β-strand 4 is a common element in the "core" of the β-sheet, but does not appear to make significant functional contributions, including the common Asp near its carboxyl end. Mutation of the homologous Asp in β, D242N, causes large defects in catalysis and the residue was proposed to stabilize the transition state (Al-Shawi and Senior, 1988; see also Senior, this issue). However, the behavior of the mutant did not support an earlier proposal (Fry *et al.*, 1986) that D242 participates in coordinating Mg^{2+}.

In AK, which has three α-helices between β-strands 4 and 5, the first helix and following loop are positioned "above" the carboxyl end of the β-sheet and contain several residues that make important contacts with the phosphoryl groups of nucleotide bound at the ATP site. In particular, AK residues R132 in the helix and R138 in the loop may interact with the phosphoryl undergoing transphosphorylation, thereby stabilizing the transition state (Tsai and Yan, 1991). In the β subunit of F_1, the number of residues between predicted β-strands 4 and 5 is similar to that

for AK (~50). Residue R246 might correspond to AK R132, and mutations R246C/H cause severe defects in multisite and unisite hydrolysis by F_1 (Noumi *et al.*, 1986b; Al-Shawi *et al.*, 1989). Residue R260 might correspond to AK R138, since it is a similar number of residues beyond the homlogous Asp of β-strand 4. Between R246 and R260, a randomly generated mutation (G251D) causes almost complete loss of membrane ATPase activity (Lee *et al.*, 1991). A predicted turn follows soon after R260, and α-helix 4 probably packs "behind" the β-sheet, in analogy to the second helix after β-strand 4 in AK and the single α-helix between β-strands 4 and 5 in Ras.

β-Strand 5 through α-Helix 5

In AK and Ras, the purine ring of bound nucleotide interacts with residues near the carboxyl end of β-strand 5 and in the following loop. Similar interactions are likely for the adenine ring at catalytic sites of F_1, since several residues in this region of β are modified by nucleotide affinity probes. Y297 is labeled during inactivation of F_1 by Nbf-Cl or 8-azido-ANP and, at elevated pH, the Nbf group transfers to K155 (Andrews *et al.*, 1984). This indicates that Y297 and K155 are in close proximity. This is supported by analogy with Ras: an Asn, positioned like Y297 near the end of β-strand 5, H-bonds to a residue in the P-loop. That Asn contributes to the guanine specificity of Ras and EF-Tu. For F_1, however, the hydroxyl and aromatic ring of Y297 are not important for nucleotide specificity or catalysis (Odaka *et al.*, 1990; Weber *et al.*, 1992). If β-strand 6 of the β subunit is parallel to β-strand 5, then predicted α-helix 5 would pack "behind" the β-sheet and would not contact the bound nucleotide.

β-Strand 6

In Ras, residues near the carboxyl end of β-strand 6 form part of the specific pocket for the guanine ring, whereas AK does not contain a sixth β-strand. Predicted β-strand 6 was not included in the original topological model for β (Duncan *et al.*, 1986), which emphasized similarities with AK, but was added by Senior (1988), based on affinity labeling of Y331 by 2-azido-ANP (Figs. 1 and 2). Studies with site-specific mutants have shown that Y331 is not essential for catalysis, but does appear to affect the purine ring specificity of the catalytic sites (Wise, 1990; Weber *et al.*, 1992).

Other Relevant Residues beyond β-Strand 6 of the β Subunit

Upon specific loading of 2-azido-ANP into noncatalytic sites of F_1, Y354 is photolabeled, indicating this residue is near the adenine ring. Mutagenic studies have shown that the hydroxyl and aromatic ring of Y354 are not essential. At pH 8, Y354 also can be labeled by FSBA, which probably adopts a "stacked" conformation that positions the reactive group near the adenine ring (Jacobson and Colman, 1984). Since the α subunits appear to confer much of the adenine specificity of the noncatalytic sites on F_1, Y354 is probably at an α–β subunit interface. This has been confirmed recently by cross-linking with a bifunctional probe, 8-azido-FSBA (Zhuo *et al.*, 1992). A subunit interfacial location of the region around Y354 is also supported by immunological studies: an epitope located within the segment D345–D380 is not accessible to monoclonal antibody in intact F_1 (Tozer and Dunn, 1987).

At pH 6, predominant labeling of mitochondrial F_1 by FSBA occurs at the equivalent of S413 (bovine H427). With the assumption that FSBA is bound in a stacked conformation for this reaction, residue 413 was also assigned as being near the adenine ring of a noncatalytic nucleotide (Bullough and Allison, 1986b). Residue 413 is in a conserved, predicted β-turn (Fig. 1), consistent with an interfacial location. Also, residue 413 is close to Y331, as shown by cross-linking with 8-azido-FSBA (Zhuo *et al.*, 1992).

SUMMARY

Analogy-based models for the catalytic site of F_1 continue to provide a useful gauge for interpreting and planning affinity labeling and mutagenesis experiments until high-resolution structures are achieved. Similar considerations also could be applied to the noncatalytic sites. A greater challenge will be to identify groups involved in the functional interactions between these nucleotide-binding domains and the other subunits of F_0F_1.

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